

Supplemental Amendment

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Confirmation No.: 9854

Filed: December 5, 2000

For: STABILIZED BIOACTIVE PEPTIDES AND METHODS OF IDENTIFICATION, SYNTHESIS AND USE

Remarks

New claims 120-122 are supported by the specification at, for example, page 22, lines 12-24.

It is respectfully submitted that new claims 120-122 are members of Group II (claims 89-97, 104, and 105) elected in the Response to Restriction Requirement mailed February 24, 2003, as they are drawn to a polypeptide comprising a bioactive peptide, a first stabilizing group, and, optionally, a second stabilizing group.

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The Examiner is invited to contact Applicant's Representatives at the below-listed telephone number if there are any questions regarding this Supplemental Amendment or if prosecution of this application may be in any way assisted or expedited thereby.

Respectfully submitted for
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By:

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**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

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Amendments to the following are indicated by underlining what has been added and striking out what has been deleted.

In the Claims

For convenience, all pending claims are shown below.

1. A recombinant method for identifying a bioactive peptide comprising:
 - (a) transforming a host cell with an expression vector comprising a tightly regulable control region operably linked to a nucleic acid sequence encoding a peptide;
 - (b) growing the transformed cell under conditions that repress expression of the peptide;
 - (c) inducing expression of the peptide in the transformed host cell; and
 - (d) determining whether expression of the peptide is inhibitory of host cell growth,wherein inhibition of host cell growth is indicative of the expression of a bioactive peptide.
61. The method of claim 1 wherein the tightly regulable control region of the expression vector comprises at least a portion of the wild-type *E. coli lac* promoter/operator region, said portion comprising auxiliary *lac* operator O3, a CAP binding region, the -35 *lac* promoter site, the -10 *lac* promoter site, *lac* operator O1, *lacZ* Shine-Dalgarno sequence and a spacer region; and wherein the transformed host cell comprises an amount of Lac repressor protein effective to repress expression of the peptide during step (b).
62. The method of claim 61 wherein the host cell is a bacterium.
63. The method of claim 62 wherein the bacterium is a gram positive bacterium.
64. The method of claim 62 wherein the bacterium is gram negative bacterium.
65. The method of claim 62 wherein the bacterium is *E. coli*.

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66. The method of claim 61 wherein the host cell is a microbial pathogen.
67. The method of claim 66 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.
68. The method of claim 61 wherein the expression vector comprising the nucleic acid sequence encoding the peptide is a first expression vector, and wherein the host cell is further transformed, prior to step (b), with a second expression vector comprising a promoter operably linked to a gene encoding a Lac repressor protein.
69. The method of claim 61 wherein the expression vector has the identifying characteristics of pLAC11 (ATCC No. 207108).
70. The method of claim 69 wherein the expression vector is pLAC11 (ATCC No. 207108).
71. The method of claim 1 wherein the host cell comprises proteases or peptidases or both.
72. The method of claim 1 wherein the host cell has not been modified to reduce or eliminate the expression of naturally expressed proteases or peptidases.
73. The method of claim 1 wherein the host cell is a prokaryote.
74. The method of claim 1 wherein the host cell is a microbial pathogen.
75. The method of claim 74 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.
76. The method of claim 1 wherein the host cell is a eukaryotic cell.

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77. The method of claim 76 wherein the eukaryotic cell is a mammalian cell.
78. The method of claim 76 wherein the eukaryotic cell is a cancer cell.
79. The method of claim 1 wherein the host cell is a protozoan.
80. The method of claim 1 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.
81. The method of claim 80 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.
82. The method of claim 81 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.
83. The method of claim 1 wherein the peptide comprises a stabilizing motif.
84. The method of claim 83 wherein the stabilizing motif comprises a hydrophilic α -helix motif.
85. The method of claim 83 wherein the stabilizing motif comprises an opposite charge ending motif.

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86. The method of claim 1 wherein the peptide comprises a randomized amino acid sequence.
87. The method of claim 86 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.
88. The method of claim 86 wherein the peptide comprises a stabilizing motif.
89. A bioactive peptide comprising a first stabilizing group comprising the N-terminus of the bioactive peptide and a second stabilizing group comprising the C-terminus of the bioactive peptide, wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-, and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa, with the proviso that when the first stabilizing group is Pro-, the second stabilizing group is not -Pro-Xaa.
90. The bioactive peptide of claim 89 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein, and glutathione reductase.
91. The bioactive peptide of claim 89 wherein the first stabilizing group is Pro-Pro- and the second stabilizing group is -Pro-Pro.
92. The bioactive peptide of claim 89 wherein at least one of the first and second stabilizing groups comprises a small stable protein.

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93. The bioactive peptide of claim 92 wherein the small stable protein is a four-helix bundle protein.
94. The bioactive peptide of claim 92 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.
95. The bioactive peptide of claim 94 wherein the small stable protein is Rop protein.
96. The bioactive peptide of claim 89 which is an antimicrobial peptide.
97. The bioactive peptide of claim 89 which is a therapeutic peptide drug.
98. A bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide.
99. A fusion protein comprising a four-helix bundle protein and a polypeptide.
100. The fusion protein of claim 99 wherein the four-helix bundle protein is Rop protein.
101. The fusion protein of claim 100 wherein the polypeptide comprises a bioactive peptide.
102. The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its C-terminus to the N-terminus of the polypeptide.
103. The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its N-terminus to the C-terminus of the polypeptide.

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104. A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Xaa-Pro- and -Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Pro-Xaa and -Pro-Pro-Xaa; and

a cleavage site immediately preceding the first stabilizing group;
wherein the second stabilizing group comprises the C-terminus of the polypeptide.

105. A polypeptide comprising:

a bioactive peptide comprising (a) a first stabilizing group selected from the group consisting of Pro, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and (b) a second stabilizing group selected from the group consisting of -Pro-, -Pro-Pro-, -Pro-Xaa- and -Pro-Pro-Xaa-; and

a cleavage site immediately following the second stabilizing group;
wherein the first stabilizing group comprises the N-terminus of the polypeptide.

106. A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately preceding the plurality of sequential uniformly charged amino acids.

107. A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately following the plurality of sequential oppositely charged amino acids.

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108. A method for using an antimicrobial peptide comprising:
covalently linking a first stabilizing group to the N-terminus of the antimicrobial peptide
and a second stabilizing group to the C-terminus of the antimicrobial peptide to yield a
stabilized antimicrobial peptide; and
contacting a microbe with the stabilized antimicrobial peptide.
109. The method of claim 108 wherein the first stabilizing group is selected from the group
consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the
second stabilizing group is selected from the group consisting of a small stable protein, -Pro,
-Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.
110. The method of claim 108 wherein the first stabilizing group is selected from the group
consisting of Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and the second stabilizing group is
selected from the group consisting of -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.
111. A method for using an antimicrobial peptide comprising:
covalently linking a plurality of sequential uniformly charged amino acids to the N-
terminus of the antimicrobial peptide and covalently linking a plurality of sequential oppositely
charged amino acids to the C-terminus of the antimicrobial peptide to yield a stabilized
antimicrobial peptide; and
contacting a microbe with the stabilized antimicrobial peptide.
112. A method for treating a patient having a condition treatable with a peptide drug
comprising administering to the patient a stabilized form of the peptide drug.
113. The method of claim 112 wherein the stabilized form of the peptide drug comprises a
first stabilizing group comprising the N-terminus of the peptide drug and a second stabilizing
group comprising the C-terminus of the peptide drug.

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114. The method of claim 113 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and -Pro-Pro-Xaa.

115. The method of claim 114 wherein the small stable protein is a four-helix bundle protein.

116. The method of claim 114 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

117. The method of claim 113 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking the first stabilizing group to the N-terminus of a peptide drug and covalently linking the second stabilizing group to the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

118. The method of claim 112 wherein the stabilized form of the peptide drug comprises an opposite charge ending motif.

119. The method of claim 118 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the peptide drug and covalently linking a plurality of sequential oppositely charged amino acids comprising the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

120. (NEW) A polypeptide comprising a bioactive peptide and a stabilizing group attached to either or both of the N-terminus or C-terminus of the bioactive peptide, wherein the stabilizing group attached to the N-terminus, if present, comprises Xaa-Pro-Pro- and the stabilizing group attached to the C-terminus, if present, comprises -Pro-Pro-Xaa.

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121. (NEW) A polypeptide comprising a bioactive peptide and a stabilizing group comprising Rop protein attached to either or both of the N-terminus or C-terminus of the bioactive peptide.

122. (NEW) A polypeptide comprising a bioactive peptide and a stabilizing group comprising a four-helix bundle protein attached to either or both of the N-terminus or C-terminus of the bioactive peptide.